IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF	Kranich et al.
SERIAL NO.	US 10/593,259
FILED:	July 26, 2007
FOR:	Non-Glycosylated/Non-Glycosidic/Non-Peptidic Small Molecule PSGL-1 Mimetics for the Treatment of Inflammatory Disorders

DECLARATION

I, Dr. Remo Kranich, born on December 28, 1969 in Hennigsdorf, Germany, having a master ("Diploma") of chemistry, a citizen of the Federal Republic of Germany and residing at Hennigsdorfer Strasse 141N in D-13503 Berlin, Germany, declare as follows:

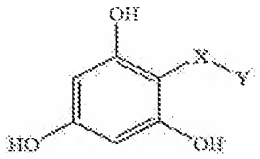
I am a fully trained chemist, having studied chemistry at the Technical University of Berlin, Germany, from 1990 to 1996; I was awarded my bachelor ("Diploma") degree by the latter university in 1996; I finalized my PhD studies in organic chemistry at the Technical University of Berlin in 1999 and held a post-doctoral Biomedical Research Associate position at The Scripps Research Institute, La Jolla, CA, United States from 2000 until 2001. Since 2001, when I joined Revotar AG at Neuendorfstrasse 24a in D-16761 Hennigsdorf, Germany, I have been engaged in the synthesis, research and development of small molecule compounds.

I am one of the inventors of the invention disclosed and claimed in Application US 10/593,259 (filed July 26, 2007), and I am therefore fully conversant with the technical area to which application 10/593,259 pertains;

I have read the application and studied the application file, in particular the Office Action dated March 16, 2011, and the prior art referenced therein, and I am therefore also well acquainted with the invention, which is disclosed and claimed in Application US 12/067,059. The following experiments and tests were carried out under my supervision in accordance with the standardized procedures as described in the patent application and in particular on page 65 of the application as originally filed (page 32, paragraph [0164] to page 33, paragraph [0165] of published US2008/0249107). I have reviewed the test protocols and based on my review and knowledge, I consider those data to be fully reliable:

The compounds of the present Application US 10/593,259, filed July 26, 2007, comprise a 2,3,4-trihydroxy substituted phenyl ring or a 3,4,5-trihydroxy substituted phenyl ring.

Contrary to this, the chemical compounds of Application US 12/067,059 (now patent US 7,919,532) encompass a trihydroxy-phenyl structure having the following specific 2,4,6 substitution pattern:



It will be shown that the structures as claimed in the Application US 10/593,259 lead to unexpected technical effects compared to the compounds of patent US 7,919,532.

The pharmaceutical activity of compounds according to the Application US 10/593,259 (e.g. compound 48 and two additional compounds that are covered by the claims of Application US 10/593,259) are compared with the activity data of the corresponding compounds of patent US 7,919,532 (compounds 63, 26, 60).

The flow chamber assay and the procedure of testing are described below (as in applications US 10/593,259 and US 12/067,059).

Flow Chamber Assay / Cell Adhesion and Rolling under Flow Conditions

To assess the capability of compounds to inhibit cell binding under dynamic conditions resembling the flow in a blood vessel, flow chamber assays addressing/ testing binding of HL-60 cells / various cell lines to P-selectin, L-selectin and E-selectin chimeric molecules are performed.

Cell attachment under flow conditions is determined using a parallel flow chamber system. A 35 mm polystyrene culture dish is coated for 1 hour at room temperature with coating buffer (50 mM tris-(hydroxymethyl) aminomethane buffer (Tris), 150 mM NaCl, 2 mM $CaCl_2$; pH 7.4) containing human E- or P-selectin-IgG chimera at concentrations of 2.5 g/mL or 10 g/mL, respectively.

After removal of the coating solution non specific binding sites are blocked for an additional hour with 1% BSA in coating buffer at room temperature. After washing with assay buffer ("Roswell Park Memorial Institute 1640" (RPMI 1640) + 10 mM HEPES) the dish is fitted into a parallel plate laminar flow chamber (sold from Glycotech, Rockville, MD) and mounted on an inverted phase-contrast microscope (sold from Olympus, Hamburg, Germany) equipped with a CCD camera (JVC) that is connected to a PC. Employing a peristaltic pump (sold from Ismatec, Wertheim-Mondfeld, Germany) the re-circulating system is equilibrated with assay buffer containing 125 µM compound or vehicle control (dimethylsulphoxide).

Cells (1 million/mL) are added to the chamber and allowed to distribute for 2 minutes at a high flow rate. The flow rate is then decreased resulting in a calculated flow shear of 1 dyne/cm^2. Video sequences of 10 low power fields are digitally recorded after 5 minutes continuous flow. The percentage of inhibition is calculated from the mean number of cells per field that attached to the coated dish surface in the presence versus absence of compound at independent experiments.

In the following Table 1, the experimental data are compared concerning the inhibition of cell binding, which are assessed with a Flow Chamber Assay as described in the present Application US 10/593,259 and also in US 12/067,059 (see US 2008/0207741).

The values of the inhibition ratios are given as normalized ratio of %-inhibition of the respective compound divided by %-inhibition of the selectin inhibition standard compound Bimosiamose (1,6-Bis [3-(3-carboxymethylphenyl)-4-(2-α-D-manno-pyranosyloxy)-phenyl] hexane):

[inhibition ratio] = [%-inhibition of compound] / [%-inhibition of standard (Bimosiamose)]

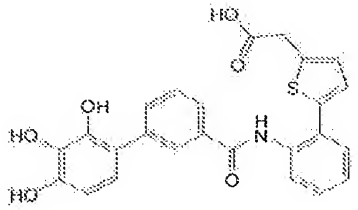
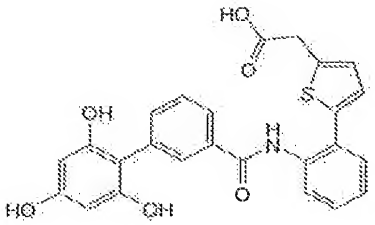
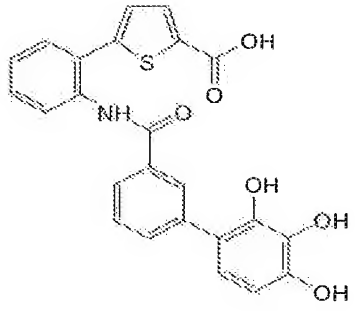
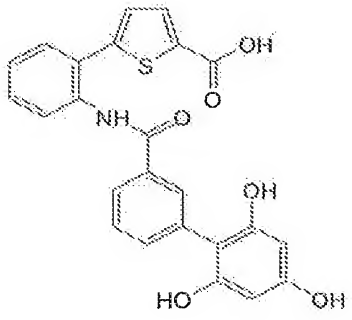
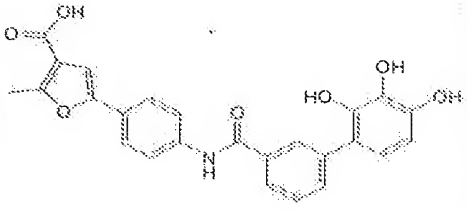
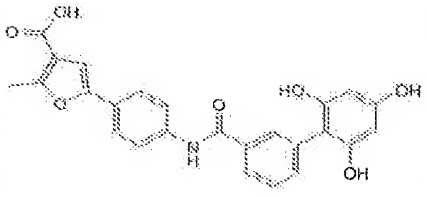
Chemical Structure	Inhibition Ratio of i) E-selectin ii) P-selectin	Chemical Structure	Inhibition Ratio of i) E-selectin ii) P-selectin
Compound example 48 of Application US 10/593,259 	i) 1.4 ii) 2.0	Compound example 63 of Application US 12/067,059 	i) 1.2 ii) 0.9
Compound according to Application US 10/593,259 	i) 2.0 ii) 2.6	Compound example 26 of Application US 12/067,059 	i) 1.1 ii) 0.8
Compound according to Application US 10/593,259 	i) 1.4 ii) 1.5	Compound example 60 of Application US 12/067,059 	i) 1.0 ii) 1.0

Table 1

The experimental test results summarized in Table 1 clearly demonstrate that the 2,3,4-trihydroxy substituted phenyl compounds and the 3,4,5-trihydroxy substituted phenyl compounds according to the Application US 10/593,259 have a higher normalized inhibition of either E- and P-selectin than the 2,4,6 trihydroxy phenyl compounds according to patent US 7,919,532.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issuing thereon.

By 

Dr. Remo Kranich Date: August 23, 2011